New Diagnostic and Therapeutic Approaches in Otologic Diseases

Guest Editors: Fatih Oghan, Cemal Cingi, Erkan Karatas, and Sebahattin Cureoglu
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Editorial

New Diagnostic and Therapeutic Approaches in Otologic Diseases

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Cochlear pathologies and vestibular disorders have been investigated in detail in the recent years. Recent advances in diagnostic and therapeutic methods have introduced new investigations about the cytologic degenerations and regenerations of cochlear cells. Knowledge and understanding of otologic diseases have led to the development of novel therapies, approaches, and/or tools.

This special issue covers the important topics in the diagnosis and therapies of otologic diseases including chronic otitis media, tinnitus, ototoxicity, and hearing loss.

Investigation of the Presence of Biofilms in Chronic Suppurative Otitis Media, Nonsuppurative Otitis Media, and Chronic Otitis Media with Cholesteatoma by Scanning Electron Microscopy. Biofilms have been shown to play a major role in the pathogenesis of otolaryngologic infections. However, very limited studies have been undertaken to demonstrate the presence of biofilms in tissues from patients with chronic otitis media (COM) with or without cholesteatoma. E. Kaya et al. found that their study supports the hypothesis that biofilms are involved in chronic suppurative otitis media, cholesteatoma, and to a lesser degree, chronic nonsuppurative otitis media. The presence of biofilms was significantly higher in the middle ear mucosa compared with the mastoid and ossicle samples. They emphasized that careful use of topical or systemic antimicrobials is essential and during surgery, hypertrophic tissue must be carefully removed from normal tissue.

Is It Necessary to Do Temporal Bone Computed Tomography of the Internal Auditory Canal in Tinnitus with Normal Hearing? Tinnitus is the perception of sound without an external stimulus. The prevalence of tinnitus varies between 3–30% of all population. As we know very well, tinnitus can be seen due to the pressure of the acoustic neuromas, cerebellopontine angle tumors, and vascular lesions, such as vascular loop to the eight cranial nerve reported in the literature. The development of the tinnitus can be observed due to nerve edema, degeneration, and compression in the canal. Accordingly, the pathological conditions that affect the width of the canal can lead to tinnitus due to compression. T. L. Kumral et al. investigated the diameter of internal acoustic canal in physiologically impaired tinnitus patients as the etiology may be due to anatomical differences of the temporal bone. In conclusion they found that there were no anatomical differences in the etiology of tinnitus rather than physiological degeneration in the nerves.

Efficacy of Low-Level Laser Therapy in the Management of Tinnitus due to Noise-Induced Hearing Loss: A Double-Blind Randomized Clinical. Several remedial modalities for the treatment of tinnitus have been proposed, but an effective standard treatment is still to be confirmed. In the present study, A. Mollasadeghi et al. investigate the effect of low-level laser therapy on tinnitus accompanied by noise-induced hearing loss. They found low-level laser therapy to be effective
in alleviating tinnitus in patients with noise-induced hearing loss, although this effect has faded after 3 months of follow-up.

The Role of Thiamine Pyrophosphate in Prevention of Cisplatin Ototoxicity in an Animal Model. Cisplatin ototoxicity has several characteristics. Inner ear toxicity is often a dose limiting side effect that hampers optimal cisplatin-based chemotherapy. It is normally manifested as a sensorineural hearing loss beginning in the high frequencies, successively progressing towards the speech frequency range. O. Kuduban et al. investigate the effectiveness of thiamine pyrophosphate against cisplatin-induced ototoxicity in guinea pigs. They found that systemic administration of thiamine pyrophosphate yielded statistically significant protection to the cochlea of guinea pigs from cisplatin toxicity. They emphasized that further experimental animal studies are essential to determine the appropriate indications and dosages of thiamine pyrophosphate before clinical use.

Extended High Frequency Audiometry in Polycystic Ovary Syndrome. Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting 5–10% of women in the reproductive age. Disease is characterized by oligo/amenorrhea, hyperandrogenism, and polycystic ovaries. PCOS is a chronic proinflammatory state. C. Kucur et al. evaluated extended high frequency hearing loss in PCOS patients. They concluded that PCOS patients have hearing impairment especially in extended high frequencies. Further studies are needed to help elucidate the mechanism behind hearing impairment in association with PCOS and to see whether the impairment of extended high frequency audiometry in these cases is progressive.

Possible Protective Effect of Sertraline against Cisplatin-Induced Ototoxicity: An Experimental Study. Cisplatin is a widely used chemotherapeutic agent but its ototoxicity side effect can occur in the majority of patients. Lots of agents were tried to prevent this, but there is not a routine treatment modality yet. The aim of this study was to evaluate the otoprotective effect of sertraline, which is an antidepressant with neuroprotective effects, against cisplatin, in rats. M. Ozturk et al. showed that sertraline seems to have a protective effect on cisplatin ototoxicity, and could be used to prevent the ototoxicity and also to treat the depression that occurred in cancer patients together.

Fatih Oghan
Cemal Cingi
Erkan Karatas
Sebahattin Cureoglu
Clinical Study

Is It Necessary to Do Temporal Bone Computed Tomography of the Internal Auditory Canal in Tinnitus with Normal Hearing?

Tolgar Lutfi Kumral, 1 Guven Yıldırım, 1 Huseyin Baki Yılmaz, 2 Seckin Ulusoy, 3 Guler Berkiten, 1 Suzan Deniz Onol, 4 Yusuf Ozturkçu, 1 and Yavuz Uyar 1

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Objective. To investigate the compression of the vestibulocochlear nerve in the etiology of the tinnitus in the normal hearing ears with temporal bone computed tomography scans. Methods. A prospective nonrandomized study of 30 bilateral tinnitus and 30 normal hearing patients enrolled in this study. Results. A total of 60 patients (ages ranged from 16 to 87) were included. The tinnitus group comprised 11 males and 19 females (mean age 49.50 ± 12.008) and the control group comprised 6 males and 24 females (mean age 39.47 ± 12.544). Regarding the right and left internal acoustic canals measurements (inlet, midcanal, and outlet canal lengths), there were no significant differences between the measurements of the control and tinnitus groups (P > 0.005). There was no narrowness in the internal acoustic canal of the tinnitus group compared with the control group. High-frequency audiometric measurements of the right and left ears tinnitus group at 8000, 9000, 10000, 11200, 12500, 14000, 16000, and 18000 Hz frequencies were significantly lower than the control group thresholds (P < 0.05). There was high-frequency hearing loss in the tinnitus group. Conclusion. There were no anatomical differences in the etiology of tinnitus rather than physiological degeneration in the nerves.

1. Introduction

Tinnitus is the perception of sound without an external stimulus. The prevalence of tinnitus varies between 3 and 30% of all population [1]. Tinnitus is a symptom of many diseases rather than a unique disease. Nonpulsatile form is more frequent in tinnitus. Tinnitus can originate from any part of the auditory system [2].

Most of the time the etiology of nonpulsatile tinnitus is not known. Hearing loss is the most frequent known etiology [3]. Symmetric hearing loss is observed in patients with tinnitus due to noise exposure. Although tinnitus is often seen in patients with hearing loss, it can also be seen in patients with normal hearing. For this reason it is not known whether tinnitus arises from the cochlea, the hearing nerve, or the central nervous system.

The severity of tinnitus varies from mild to severe and can be bad enough to interfere with a person’s daily activities even leading to distress, depression, and suicide by reducing the quality of life [4].

In order to find out the etiology of tinnitus, a good history, physical examination, radiological diagnosis, and audiological examinations are very important. Many systemic diseases such as hyperthyroidism, hypertension, and hypercholesterolemia are proven in the etiology, but the pathophysiology is still unknown [5].

As we know very well, tinnitus can be seen due to the pressure of the acoustic neuromas, cerebellopontine angle tumors, and vascular lesions, such as vascular loop to the right cranial nerve reported in the literature. The development of the tinnitus can be observed due to nerve edema, degeneration, and compression in the canal. Accordingly, the
2. Material and Methods

This study was performed in 30 bilateral tinnitus patients who were referred to the outpatient clinic and 30 patients without any ear disease between 2011 and 2012. Microscopic ear examination and a complete audiological examination were performed. The study group had no symptoms and signs other than tinnitus. In the physical examination, they had normal external ear canal and tympanic membrane. Patients with normal hearing thresholds in audiometric tests at the octave frequencies of 250–4,000 Hz were included in the study group. The patients with any ear complaints other than tinnitus such as chronic serous otitis media, chronic otitis media, trauma history, and external ear problems were excluded from the study. Also the patients with hyperlipidemia, hypertension, hyperthyroidism, and other systemic diseases which may cause vestibular toxicity were excluded.

Both tinnitus and control groups had high-frequency audiogram at 8000, 9000, 10000, 11200, 12500, 14000, 16000, 18000 and 20000 Hz frequencies. All patients had temporal bone computed tomography imaging. The internal auditory canal inlet, mid-canal, and outlet canal lengths were measured at the most distinctive cross-section of the seventh and eighth cranial nerves bifurcation (Figure 1). Patients who were admitted to our outpatient clinic other than ear disease with the temporal bone computed tomography results were taken as control group. Informed consent and ethical approval have been taken from all the participants. Measurements of internal auditory canal inlet, mid-canal, and outlet canal lengths were compared between the groups.

2.1 Statistical Analysis. In the statistical model, gender (male/female), age group, measurements of internal acoustic canal, and frequencies (250, 500, 1000, 2000, 4000, 8000, 9000, 10000, 11200, 12500, 14000, 16000, 18000, and 20000 Hz) were evaluated as the main factors. For statistical analysis, SPSS 17.0 V software was used to assess the findings of the study. Descriptive statistical methods (mean, standard deviation) as well as Student’s t-test for the comparison of quantitative data showing the parameters of the normal distribution were used for the determination of difference between the groups. The significance levels were set as $P < 0.05$ and 95% confidence interval.

3. Results

A total of 60 patients were included in this study. The ages ranged from 16 to 87. The tinnitus group comprised 11 males and 19 females (mean age $49,50 \pm 12,008$) and control group comprised 6 males and 24 females (mean age $39,47 \pm 12,544$) (Table 1). Tinnitus and the control group did not differ significantly by gender ($P = 0, 152$) (Table 2).

Regarding the right and left internal acoustic canals measurements (inlet, mid-canal, and outlet canal length), there were no significant differences between the measurements of the control and tinnitus groups ($P > 0.005$) (Table 3).

Tinnitus group was evaluated according to internal acoustic canal measurements and there was no significant difference between the right and left canals ($P > 0.05$) (Table 4).

High-frequency audiometric measurements of the right and left ear tinnitus group at 8000, 9000, 10000, 11200, 12500, 14000, 16000, and 18000 Hz frequencies were significantly lower than the control group thresholds ($P < 0.05$). There was significant decrease at 20000 Hz frequency in the control group ($P < 0.05$) (Table 5).

4. Discussion

Every nerve fiber has an electric discharge, even at rest, where this represents the spontaneous activity of the nerve. In patients with tinnitus, there is an increase in this spontaneous activity. As a result, hyperactive cilia or hyperactive nerve fibers may appear and the nerve fibers perceive sounds that cannot be heard under normal conditions within central auditory and nonauditory structures [6, 7]. This could explain the persistence of tinnitus after total hearing amputation.
Table 3: Comparison of measurements of the internal auditory canal of the tinnitus and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>Std. error mean</th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right canal inlet</td>
<td>Tinnitus</td>
<td>53,30</td>
<td>12,609</td>
<td>2,302</td>
<td>−0,333</td>
<td>0,741</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>54,37</td>
<td>12,235</td>
<td>2,234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right mid-canal</td>
<td>Tinnitus</td>
<td>46,67</td>
<td>10,933</td>
<td>1,996</td>
<td>1,067</td>
<td>0,291</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>43,93</td>
<td>8,800</td>
<td>1,607</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right canal outlet</td>
<td>Tinnitus</td>
<td>29,03</td>
<td>5,055</td>
<td>0,923</td>
<td>0,510</td>
<td>0,612</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>28,00</td>
<td>9,879</td>
<td>1,804</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right canal length</td>
<td>Tinnitus</td>
<td>80,13</td>
<td>14,178</td>
<td>2,589</td>
<td>−0,156</td>
<td>0,877</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80,70</td>
<td>14,042</td>
<td>2,564</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left canal inlet</td>
<td>Tinnitus</td>
<td>58,10</td>
<td>14,320</td>
<td>2,614</td>
<td>0,282</td>
<td>0,779</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>56,93</td>
<td>17,546</td>
<td>3,203</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left mid-canal</td>
<td>Tinnitus</td>
<td>45,37</td>
<td>8,739</td>
<td>1,596</td>
<td>−0,794</td>
<td>0,431</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>47,13</td>
<td>8,496</td>
<td>1,551</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left canal outlet</td>
<td>Tinnitus</td>
<td>30,73</td>
<td>6,253</td>
<td>1,142</td>
<td>−0,851</td>
<td>0,398</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>32,73</td>
<td>11,255</td>
<td>2,055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left canal length</td>
<td>Tinnitus</td>
<td>77,43</td>
<td>14,330</td>
<td>2,616</td>
<td>−0,823</td>
<td>0,414</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80,60</td>
<td>15,453</td>
<td>2,821</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Independent samples t-test.

Table 4: The difference between measurements of the right and left internal acoustic canal.

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right-left canal inlet</td>
<td>0,734</td>
<td>0,466</td>
</tr>
<tr>
<td>Right-left mid-canal</td>
<td>0,509</td>
<td>0,613</td>
</tr>
<tr>
<td>Right-left canal outlet</td>
<td>−1,378</td>
<td>0,174</td>
</tr>
<tr>
<td>Right-left canal length</td>
<td>−1,158</td>
<td>0,252</td>
</tr>
</tbody>
</table>

*Independent samples t-test.

Tinnitus is classified as objective tinnitus (tinnitus can be heard) and subjective tinnitus which is perceived by the patient.

Today, the aim of the subjective tinnitus therapy is to increase the tolerance with sound enrichment or cognitive behavior therapy [8, 9]. Sound maskers, tinnitus-retraining therapy, and cognitive behavioral therapy are applied to relieve the symptoms caused by tinnitus. However, there is no exact solution in the treatment of tinnitus.

We investigate the anatomic reasons in the etiology of the tinnitus in the normal hearing ears. While the young population has usually normal hearing in the studies with tinnitus, hearing loss was found increasingly in the elderly. This also proves the nerve degeneration in the auditory pathways. But why are these not seen in everyone? So we evaluated the anatomy of the temporal bone by selecting patients without systemic diseases to find out if there was an anatomic difference in the tinnitus etiology.

Patients in the study group had bilateral tinnitus. Most of the patients cannot describe the localization, the time, the duration, and the severity of the tinnitus. Females were more in the tinnitus group, consistent with the literature. There was no statistically gender difference between the tinnitus group and selected control group accordingly (P > 0,05).

Vestibulocochlear nerve and the facial nerve enter the temporal bone through the internal auditory canal. The width of the internal acoustic canal varies from person to person. The etiology of tinnitus was influenced by many factors. But the pathophysiology is still unknown. Sometimes, all
the electrophysiological studies of patients suffering from
 tinnitus are normal.

 There were studies that showed marked improvement in
tinnitus when the pathologies such as vascular loop, cere-
bellopontine angle tumors, and cholesterolomas were removed
[10, 11]. So compression in the internal acoustic canal actually
causes tinnitus.

 Tinnitus and episodic vertigo attacks were also improved
after microvascular decompression of the vascular loop in
the internal auditory canal [12, 13]. This shows that nerve
compression is effective in the etiology of tinnitus.

 Meningiomas represent 3% to 12% of the tumors in the
cerebellopontine angle and may be presented with tinnitus
due to compression [14]. Exocytoses and osteomata are
benign bony lesions of the auditory canal and they can also
cause tinnitus [15].

 In cases of transverse fractures of the temporal bone, the
labyrinth is involved more frequently than in longitudinal
fractures. This may cause hearing loss and tinnitus [16].

 There have been attempts to establish relation between
vestibulocochlear nerve compression site and the character
of symptoms. But there is still no definitive data [7, 17].

 For this purpose, the internal auditory canal diameters
(inlet, mid-canal, and outlet canal lengths) were evaluated
in this study. We investigated whether narrowing in any of
these locations may be the cause of tinnitus. The results
showed that there were no significant differences in the
measurements of internal canal between control and tinnitus
groups (P < 0.05). Also comparison of the measurements
within the tinnitus groups did not show any significant differ-
ence in the right and left internal acoustic canals (P < 0.05).

 Normal hearing thresholds can be seen in tinnitus. How-
ever, normal hearing thresholds do not necessarily indicate
the absence of cochlear damage. The state of cochlea can be
judged with audiogram. Therefore, cochlear damage in study
group was evaluated with high-frequency audiogram. There
was a significant decrease in high-frequency audiometry at
8000, 9000, 10000, 11200, 12500, 14000, 16000, and 18000 Hz
frequencies even if normal audiograms at 500, 1000, 2000,
and 4000 Hz in the tinnitus group (P < 0.05). This showed
neural degeneration rather than an anatomical variation or
nerve compression in the internal auditory canal. Temporal
bone tomography of tinnitus patients with normal hearing at
the speech frequencies (500, 1000, 2000, and 4000 Hz) did not
give any additional information.

 Temporal bone imaging allows fine depiction of labyrinth
abnormalities related to neoplastic, inflammatory, ischemic,
degenerative, or traumatic disorders [13, 18]. Magnetic reso-
nance imaging is the best for soft tissue masses and intracra-
inal evaluation. However, this study shows that, in normal
hearing patients with tinnitus, temporal bone imaging did not
give any valuable information regarding tinnitus.

5. Conclusion

As a conclusion, if there is a complaint of tinnitus in patients
with normal hearing, temporal bone tomography does not
give us valuable information and it is not cost-effective to
perform it. However, if tinnitus is accompanied by hearing
loss, there may be underlying acoustic neuromas or Meniere's
disease. Therefore, a CT or MRI can be performed in all hear-
ring loss patients. It was concluded that tinnitus occurred
in patients with normal hearing due to pathophysiological
degeneration other than anatomical variations in internal
acoustic canal.

Conflict of Interests

None of the authors have any conflict of interests.

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cranial nerve. Can the site of compression explain the symp-

in the internal auditory canal (IAC): review of the literature and


Clinical Study

Efficacy of Low-Level Laser Therapy in the Management of Tinnitus due to Noise-Induced Hearing Loss: A Double-Blind Randomized Clinical Trial

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Background. Several remedial modalities for the treatment of tinnitus have been proposed, but an effective standard treatment is still to be confirmed. In the present study, we aimed to evaluate the effect of low-level laser therapy on tinnitus accompanied by noise-induced hearing loss. Methods. This was a double-blind randomized clinical trial on subjects suffering from tinnitus accompanied by noise-induced hearing loss. The study intervention was 20 sessions of low-level laser therapy every other day, 20 minutes each session. Tinnitus was assessed by three methods (visual analog scale, tinnitus handicap inventory, and tinnitus loudness) at baseline, immediately and 3 months after the intervention. Results. All subjects were male workers with age range of 30–51 years. The mean tinnitus duration was 1.85 ± 0.78 years. All three measurement methods have shown improved values after laser therapy compared with the placebo both immediately and 3 months after treatment. Laser therapy revealed a U-shaped efficacy throughout the course of follow-up. Nonresponse rate of the intervention was 57% and 70% in the two assessment time points, respectively. Conclusion. This study found low-level laser therapy to be effective in alleviating tinnitus in patients with noise-induced hearing loss, although this effect has faded after 3 months of follow-up. This trial is registered with the Australian New Zealand clinical trials registry with identifier ACTRN12612000455864.

1. Introduction

Tinnitus is defined as a sound in the ear(s) without any external auditory stimulus. About 15% of the general population experience at least one episode of tinnitus, which prevalence increases by age and reaches 85% in individuals older than 60 years [1]. This symptom is intolerable in nearly 20% of the cases [2]. Reaching as high as 67%, tinnitus is more prevalent among individuals suffering from hearing disorders [3].

Noise has such deleterious effects on hearing as noise-induced hearing loss (NIHL) is the second most common form of acquired hearing loss. It has long been recognized as a problem in noisy environments workers [4]. As a possible complication of NIHL, tinnitus is usually observed at frequencies equal to or higher than 3000 Hz, which is one octave band higher than the frequencies affected in NIHL. Its intensity is usually between 3 and 5 dB (occasionally up to 15 dB) [5].
Tinnitus may lead to such complications as depression, irritability, sleep disorders, and loss of concentration [6]. Although lacking a widely accepted treatment, various therapeutic modalities have been proposed thus far, including medications (such as sedatives, antiepileptics, antidepressants, antipsychotics, local anesthetics, antihistamines, and botulinum toxin A) [7], repetitive transcranial magnetic stimulation [8], transcutaneous electrical stimulation [9], and sound therapy [10]. Low-level laser therapy (LLLT) has recently been tried with promising results in outpatients with subjective tinnitus [2].

As known, laser has different usages in medicine such as wound healing, nerve and tissue repairing, pain control [11], and treating Meniere’s disease and tinnitus [12]. Although the exact mechanism of the effect of LLLT on tinnitus is not clearly understood, it has been proposed that it may be induced by increasing cell proliferation, growth factor secretion, improvement in inner ear blood flow, and/or activation of the hair cells mitochondria [2]. There is still some degree of controversy concerning the efficiency of LLLT in tinnitus. Some studies have shown positive effects [2, 11, 13, 14], but others have found no such effectiveness [15, 16].

Considering the fact that NIHL is a common disorder in industrial settings and tinnitus is its most common associated subjective complaint, we designed an interventional study to evaluate the effect of LLLT on tinnitus accompanied by NIHL.

2. Methods

2.1. Study Design and Population. The present study was a double-blind randomized clinical trial with the participation of patients referred to the occupational medicine clinic of Shahid Sadoughi University of Medical Sciences. Recruitment took place from September 2010 till September 2011.

One hundred volunteers younger than 50 years suffering from NIHL (defined as a bilateral sensorineural hearing loss, with the hearing threshold higher than 15 dB at least at one of the following frequencies: 3000, 4000, and 6000 Hz [4]) and tinnitus have enrolled to the study. The level of effect observed in a former study was used for the calculation of the sample size [2].

After baseline screening interview and examination, eleven participants were excluded from the study, yielding a final sample size of 89. Our main exclusion criteria were as follows: any history of exposure to ototoxic drugs/substances, psychotic disorders with auditory hallucination, acoustic trauma, head trauma, mumps, meningitis, Meniere’s disease, and having any contraindication for laser therapy [17].

Subjects were randomly allocated to either laser therapy or placebo groups. Randomization was done using a random digit table. According to the principles of double blindness, the study participants and operators who performed the assessment tests as well as the researchers who evaluated the outcomes were completely blinded to the groups.

After taking a thorough medical and occupational history, the microscopic examination of auditory meatus and tympanic membrane was performed. Afterwards, subjects underwent pure-tone audiometry performed at 250, 500, 1000, 2000, 3000, 4000, 6000, and 8000 Hz frequencies (device: clinical audiometer, Interacoustic, AC40; headphone: TDH39, Denmark) in an acoustic chamber meeting the American National Standards Institute criterions [18]. Tympanometry was also accomplished for all participants (device: Tympanometer, Interacoustic, AZ26, Denmark). Subjects in the intervention group underwent laser therapy for 20 sessions, every other day, 20 minutes each session, which was a combination of protocols used in the previous studies [1, 2, 13]. A low-level laser beam with wave length of 650 nm and intensity of 5 mW was irradiated to the ear via mastoid bone (device: TINNImed, Switzerland). This device was connected to the ear by a soft silicone tip. The treatment sessions were performed for the subjects in placebo group with turned-off device.

A written informed consent was obtained from all participants before the enrolment. The protocol of the study was approved by the ethics committee of research vice chancellor of Shahid Sadoughi University of Medical Sciences.

2.2. Efficacy Assessments. We used the following three validated methods for the evaluation of outcome before treatment, immediately and 3 months after the termination of treatment: tinnitus visual analog scaling (VAS), tinnitus handicap inventory (THI), and tinnitus loudness measurement. Visual analog scale is scored on a 10-point scale, in which individuals select the lowest perceived loudness on a scale of 0 to 10 corresponding to an increasing level of loudness [19]. In THI scoring, 25 questions are asked from the patient and the severity of tinnitus is categorized as follows. Grade 1 (0–16): Slight (only heard in quiet environments); Grade 2 (18–36): Mild (easily masked by environmental sounds and easily forgotten with activities); Grade 3 (38–56): Moderate (noticed in the presence of background noise, although daily activities can still be performed); Grade 4 (58–76): Severe (almost always heard, leads to disturbed sleep patterns and can interfere with daily activities); Grade 5 (78–100): Catastrophic (Always heard, disturbed sleep patterns, difficulty with any activities) [20]. We used a translated version of the questionnaire into Persian, which was reviewed and modified by three experts to adapt our population culture. Loudness and frequency of tinnitus was assessed by audiometer. Pitch was matched by introducing two successive tones to the ear and the patient chose which one was closest to the tinnitus pitch. The loudness was assessed by matching it with the loudness of pure tone at each frequency in the contralateral ear according to the patient’s sensation.

2.3. Statistical Analysis. Data were analyzed by the Statistical Package for Social Sciences software version 15.0 (SPSS Inc, Chicago, Illinois, USA). We used independent-sample t-test for the comparison of mean tinnitus loudness between two groups in three occasions (baseline, immediately, and 3 months after intervention), and paired t-test for the comparison of treatment effect within each group in different occasions. Chi square test was also employed in the comparison of VAS and THI score changes between two groups.
3. Results

From 100 patients screened, 89 individuals were eligible for enrolment. Reasons for exclusion were as follows: exposure to ototoxic substances (n = 6), head injury (n = 2), consumption of ototoxic drug (n = 1), head trauma (n = 1), and childhood infection (n = 1). Figure 1 shows the flow diagram of the study. As demonstrated, 3 laser therapy- and 4 placebo-assigned participants have discontinued the trial due to personal reasons. Notably, no case of LLLT-attributable side effects was observed in our course of study.

All cases were males with age range of 30 to 51 years (mean: 41.17 ± 5.89 years). Their mean duration of employment was 12.21 ± 1.77 years. Mean level of noise in the workplace (time weighted average for an 8-hour shift) was 87.60 ± 1.49 dBA. Tinnitus was bilateral in 49% of the cases, while 27 and 24 percent of subjects suffered from unilateral tinnitus in left and right ears, respectively. The mean tinnitus duration was 1.85 ± 0.78 years. As expected, there was not significant difference in terms of age (P = 0.88), employment duration (P = 0.83), workplace noise level (P = 0.78), and duration of tinnitus (P = 0.62) between two randomized study groups.

Participants were categorized based on the level of experienced changes in the severity of tinnitus quantified by VAS.
Table 1: Comparison of changes in tinnitus visual analog scaling and tinnitus handicap inventory scores immediately and 3 months after intervention between groups.

<table>
<thead>
<tr>
<th>Variable [number (%)]</th>
<th>Immediately after intervention</th>
<th>3 months after intervention</th>
<th>P value</th>
<th>Laser therapy group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual analog scale score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No difference</td>
<td>22 (54)</td>
<td>35 (85)</td>
<td></td>
<td>29 (70)</td>
<td>40 (97)</td>
<td>0.003</td>
</tr>
<tr>
<td>&lt;50% reduction</td>
<td>7 (17)</td>
<td>3 (7.5)</td>
<td>0.006</td>
<td>5 (13)</td>
<td>1 (3)</td>
<td>0.003</td>
</tr>
<tr>
<td>≥50% reduction</td>
<td>12 (29)</td>
<td>3 (7.5)</td>
<td>7 (17)</td>
<td>0 (0)</td>
<td>&lt;50% reduction</td>
<td>2 (6)</td>
</tr>
<tr>
<td>≥50% reduction</td>
<td>18 (43)</td>
<td>4 (10)</td>
<td>13 (31)</td>
<td>1 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tinnitus handicap inventory score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No difference</td>
<td>21 (51)</td>
<td>36 (87)</td>
<td></td>
<td>27 (66)</td>
<td>40 (97)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt;50% reduction</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>0.001</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥50% reduction</td>
<td>18 (43)</td>
<td>4 (10)</td>
<td>13 (31)</td>
<td>1 (3)</td>
<td>&lt;50% reduction</td>
<td>2 (6)</td>
</tr>
</tbody>
</table>

Table 2: Comparison of tinnitus loudness between two groups at baseline, immediately and 3 months after intervention.

<table>
<thead>
<tr>
<th>Tinnitus loudness (dB)</th>
<th>Placebo</th>
<th>Laser therapy group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.09 ± 1.11</td>
<td>6.07 ± 1.12</td>
<td>0.922</td>
</tr>
<tr>
<td>Immediately after intervention</td>
<td>5.97 ± 1.03</td>
<td>4.51 ± 1.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 months after intervention</td>
<td>6.02 ± 1.15</td>
<td>5.09 ± 1.90</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± standard deviation.*

Table 3: Comparison of the changes of tinnitus loudness in 3 periods of assessment within and between groups.

<table>
<thead>
<tr>
<th>Tinnitus loudness (dB)</th>
<th>Placebo</th>
<th>Laser</th>
<th>P value for between-group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.09 ± 1.11</td>
<td>6.07 ± 1.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Immediately after intervention</td>
<td>5.97 ± 1.03</td>
<td>4.51 ± 1.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 months after intervention</td>
<td>6.02 ± 1.15</td>
<td>5.09 ± 1.15</td>
<td>0.01</td>
</tr>
</tbody>
</table>

and total THI. Table 1 summarizes the results of between-group analyses of distribution of changes in different time intervals. As shown, LLLB was significantly more effective than placebo immediately and 3 months after treatment, which points to the efficacy of the study intervention. Nevertheless, tinnitus severity remained unchanged in 54% and 70% of patients immediately and 3 months after receiving LLLB (as measured by VAS).

According to Table 2, tinnitus loudness scores were comparable between two groups at baseline. After receiving LLLB, tinnitus loudness score was diminished in a U-shape manner with significantly lower scores than placebo in all time points.

Changes in tinnitus loudness score were compared within and between groups in different time periods. As expressed in Table 3, LLLB reduced the loudness of tinnitus significantly in relation to the baseline values and compared with the placebo group in all time periods. A meaningful response was also detected in placebo-assigned individuals immediately after treatment, which still was significantly lower than that of the intervention group.

4. Discussion

Previously published studies have reported the efficacy of LLLT in decreasing tinnitus to be between 15-67% [11]. Quantifying by VAS, our positive findings have been multiplied by some [2, 11, 13], while negated by other studies [15, 21]. Tauber et al. used 10 sessions of LLLT with two different wavelengths (635 and 839 nm) during two weeks which was different from our practice [11]. Okhovat et al. were treated patients with 20-minute sessions a day for 20 days using the same wavelengths to our study [2]. The most similar protocol to ours was used by Yıldırım et al., with considerable improvements which sustained after two months [13].

Mixed results were also obtained by studies that have used tinnitus loudness score as their primary outcome measure. While this study in line with Tauber et al. [11], Gungor et al. [22], Newman et al. [20], and Shiomi et al. [23] has found tinnitus loudness to be improved after LLLT, two evaluations have failed to show the same efficacy [15, 16]. A noteworthy point to consider is the pronounced improvement reported by our patients after receiving placebo, which vanished at the
Table 4: Comparison of the design and results of some relevant studies with the present study.

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Number of cases</th>
<th>Wave length</th>
<th>Laser properties</th>
<th>Duration of treatment</th>
<th>Placebo controlled</th>
<th>Followup</th>
<th>Tinnitus scoring method</th>
<th>Effect of laser in treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>82</td>
<td>650</td>
<td>5</td>
<td>20 min, 3 days a week, for 20 sessions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Pos.</td>
</tr>
<tr>
<td>Shiomi et al. (1997)</td>
<td>38</td>
<td>830</td>
<td>40</td>
<td>9 min a week for 10 sessions</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Pos.</td>
</tr>
<tr>
<td>Mirz et al. (1999)</td>
<td>59</td>
<td>830</td>
<td>50</td>
<td>10 min per session</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>Nakashima et al. (2002)</td>
<td>45</td>
<td>60</td>
<td></td>
<td>Once a week for 4 weeks</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Neg.</td>
</tr>
<tr>
<td>Prochazka (2002)</td>
<td>72</td>
<td>830</td>
<td>300</td>
<td>Twice a week for 5 weeks and after 2-3 m. 5-6 sessions in a week</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Pos.</td>
</tr>
<tr>
<td>Tauber et al. (2003)</td>
<td>35</td>
<td>G1: 635</td>
<td>G2: 830</td>
<td>5 days a week for 2 weeks</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Pos.</td>
</tr>
<tr>
<td>Gungor et al. (2008)</td>
<td>45</td>
<td>650</td>
<td>5</td>
<td>15 min a day for 1 week</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Pos.</td>
</tr>
<tr>
<td>Cuda and De Caria (2008)</td>
<td>46</td>
<td>650</td>
<td>5</td>
<td>20 min a day for 3 months</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Pos.</td>
</tr>
<tr>
<td>Teggi et al. (2009)</td>
<td>60</td>
<td>650</td>
<td>5</td>
<td>20 min a day for 3 months</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>Okhovat et al. (2011)</td>
<td>61</td>
<td>650</td>
<td>5</td>
<td>20 min a day for 20 days</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Pos.</td>
</tr>
<tr>
<td>Yıldırım et al. (2011)</td>
<td>30</td>
<td>650</td>
<td>5</td>
<td>20 min 5 times a week for 8 weeks</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Pos.</td>
</tr>
</tbody>
</table>
end of the follow-up period. This observation, to our opinion, might be explained best by a placebo effect.

The results of total THI score in our study were in accordance with VAS results after LLLT and were consistent with the study of Cuda and Caria [14], but Teggi et al. did not show this change [15]. Table 4 presents a detailed comparison between the findings of some relevant studies with what we found in our population. We suppose that the controversial results could be attributable to employing different treatment courses, as well as varied experiment settings. For instance, our patients received therapy in clinic, while Teggi et al. [15] gave the participants their course of treatment at home.

Even though it remained higher compared with the baseline level and placebo group, we observed that the effect of LLLT attenuated after 3 months. Our finding was attenuated by another research with assessment period of 4 weeks and 6 months [11]. It seems that the efficacy of LLLT decreases over time, which may necessitates repeating the therapy. Further evidence, however, is needed for determining a proper time interval between sessions.

While most of the former comparable studies have not taken concomitant hearing disorders into consideration, we assessed the effect of LLLT on tinnitus in a background of sensorineural hearing loss. However, our results should be interpreted in the light of some limitations. The first limitation was our 3-month follow-up period that made it impossible to evaluate long-term outcomes of the studied intervention. Secondly, due to the fact that our study population comprised of male workers, the obtained results may hardly be generalized to other populations.

In conclusion, this study has provided evidence for the efficacy of LLLT in relieving NIHL accompanied tinnitus, an effect that was weakened after 3 months follow-up. Despite significant improving results, the LLLB treatment nonresponse rate was considerable which should be taken into account when considering this treatment method.

Conflict of Interests

The authors declare that there is no conflict of interests that would prejudice the impartiality of this work.

Acknowledgment

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References


Research Article

Investigation of the Presence of Biofilms in Chronic Suppurative Otitis Media, Nonsuppurative Otitis Media, and Chronic Otitis Media with Cholesteatoma by Scanning Electron Microscopy

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Objective. Biofilms have been shown to play a major role in the pathogenesis of otolaryngologic infections. However, very limited studies have been undertaken to demonstrate the presence of biofilms in tissues from patients with chronic otitis media (COM) with or without cholesteatoma. Our objective is to study the presence of biofilms in humans with chronic suppurative and nonsuppurative otitis media and cholesteatoma.

Study Design. In all, 102 tissue specimens (middle ear, mastoid tissue, and ossicle samples) were collected during surgery from 34 patients.

Methods. The samples were processed for the investigation of biofilms by scanning electron microscopy (SEM).

Results. Our research supports the hypothesis in which biofilms are involved in chronic suppurative otitis media, cholesteatoma, and, to a lesser degree, chronic nonsuppurative otitis media. There were higher rates in hypertrophic and granulated tissue samples than in normal mucosa. In addition, the presence of biofilms was significantly higher in the middle ear mucosa compared with the mastoid and ossicle samples. Conclusion. In the clinic, the careful use of topical or systemic antimicrobials is essential, and, during surgery, hypertrophic tissue must be carefully removed from normal tissue.

1. Introduction

Biofilms are complex bacterial communities that adhere to the surface of implanted biomaterial or mucosa [1]. They are embedded in a slim-like extracellular matrix composed of proteins, polysaccharides, and nucleic acids known as extracellular polymeric substances (EPS) [2]. Because they have effective defense mechanisms against the immune system of their host and against antimicrobial agents, they are difficult to eradicate [3, 4].

Today, the diagnosis, treatment, and prevention of biofilm infections clearly require different strategies from those used against acute infections [5]. In particular for mucosal biofilms, we need to better understand the interaction between the bacterial attachment and the human host [6]. The altered microenvironment in the mucosa and the degree of colonization are also important [7].

The importance of biofilms in otolaryngologic infections is becoming increasingly apparent [8]. At present, much of the literature on this subject involves in vitro studies, with the majority related to complications involving medical implants [9]. Recently, a number of publications have shown the presence of biofilms on the mucosal surfaces of tonsils and adenoids. Biofilm has also been demonstrated in otitis media with effusion and direct biopsy specimens of the middle ear mucosa and in a nonhuman primate model of chronic otitis media [10]. Biofilms are nearly impossible to detect with standard culture techniques [11] because these techniques do not elucidate the complex, three-dimensional aspects of biofilms. Molecular diagnostics based nucleic acid upon amplification strategies have provided the means to be detected and identify bacteria, and various imaging technologies have given researchers insights into the role of biofilms in human
Table 1: The biofilm findings according to the specimen distribution in patient groups.

<table>
<thead>
<tr>
<th>Pt</th>
<th>ME a</th>
<th>CSOM</th>
<th>CNSOM</th>
<th>Cholesteatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt</td>
<td>ME a</td>
<td>CSOM</td>
<td>CNSOM</td>
<td>Cholesteatoma</td>
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<td>13</td>
</tr>
</tbody>
</table>

aMiddle ear mucosa; b mastoid mucosa; c ossicle samples.

infections [12]. Scanning electron microscopy (SEM) is also an advanced resolution method that provides ultrastructure analysis of biofilms [13]. Our objective is to study the presence of biofilm in humans with chronic otitis media with or without cholesteatoma.

2. Materials and Methods

Patients undergoing surgical treatment were asked to participate in our study. The study was approved by the ethics committee of the Faculty of Medicine of Eskisehir Osmangazi University. The tissue samples were collected during routine surgical treatment from 34 patients in the Eskisehir Osmangazi University Medical Faculty during the period between October 2011 and May 2012. These patients included 16 females and 18 males. The chronic otitis media (COM) patients were divided into three groups: chronic suppurative otitis media (CSOM) (n = 10, 30 specimens); chronic non-suppurative otitis media (CNSOM) (n = 11, 33 specimens); and chronic otitis media with cholesteatoma (n = 13, 39 specimens). Various tissue samples from the patients in each group were harvested including from the middle ear mucosa, mastoid tissue, and ossicle. In addition, during the surgery, the middle ear mucosa was classified as normal, hypertrophic, or granulated tissue with associated mucosa. Tissue was taken only if the debridement of the tissue was necessary during the surgical treatment. Any eroded ossicle that could not be used for reconstruction was also removed and evaluated for biofilm formation.

Our cases with cholesteatoma represented acquired cholesteatoma cases, and they were divided into three groups according to the location of the tissue: attic (A), sinus (S), and pars tensa (PT) [14].

The tissue samples were immediately placed in 2.5% glutaraldehyde (prepared in 0.1 M phosphate buffer, pH 7.4) for 24 hours at 4 °C as a prefixation step. They were then rinsed twice with 0.1 M phosphate buffer (pH 7.4), postfixed using 1% osmium tetroxide for 1 hour at room temperature, and finally rinsed with distilled water. Next, the specimens were dehydrated using graduated concentrations of ethyl alcohol (30%, 50%, 70%, 90%, and 96%) for 15 minutes each followed by absolute alcohol for 30 minutes. The specimen was dried using the critical point dryer Polaron CPD 7501 Critical Point Dryer (VG. Microtech, East Sussex, UK). For mounting, carbon conductive paint was used; for specimens, gold coating with Polaron SC7620 Sputter Coater was used. Finally, each specimen was examined using a JEOL scanning electron microscope (JEOL JSM-5600LV). Several areas of each sample were systematically scanned. A sample was considered to have a biofilm if 3 criteria were met: (1) presence of bacterial-sized and -shaped objects; (2) presence of an amorphous material, consistent with glycocalyx around the bacteria; and (3) surface binding [15, 16].

3. Results

A total of 102 specimens were collected from 34 patients. The mean age of patients was 40.8 years for the CSOM group, 34.7 ± 11.6 years for the CNSOM group, and 28 ± 23.7 years for the cholesteatoma group. Of the 10 CSOM patients, biofilm formation was observed in 7 (70%) cases by SEM. In the CNSOM group, 6 of 11 (54.5%) patients showed a biofilm. Eight (61.5%) of the 13 patients with cholesteatoma had a biofilm (Table 1). The biofilm findings according to the specimen distribution were presented in Table 1. Among tissue samples obtained from the three-patient groups, biofilm formation was the most frequently observed in the middle ear mucosa samples (50% in CSOM group, 54.5% in the CNSOM group, and 38.4% in the cholesteatoma group). During the surgery, intraoperative cases of biofilm-positive samples were evaluated, with the results presented in Table 2. We found that the biofilm rate was higher in hypertrophic and granulated tissue than in normal mucosa. In the cholesteatoma cases, the biofilm conditions depending on the location are presented in Table 3. Additionally, because the number of biofilm-positive samples was low in this group,
Table 2: The intraoperative condition of biofilm positive samples during the surgery.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Diagnosis/condition</th>
<th>Description of specimen</th>
<th>The intraoperative condition of biofilm positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal mucosa</td>
</tr>
<tr>
<td>10</td>
<td>Chronic suppurative otitis media</td>
<td>Middle ear mucosa</td>
<td>1 (20%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastoid tissue</td>
<td>1 (33.7%)</td>
</tr>
<tr>
<td>11</td>
<td>Chronic nonsuppurative otitis media</td>
<td>Middle ear mucosa</td>
<td>1 (17%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastoid tissue</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Cholesteatoma</td>
<td>Middle ear mucosa</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastoid tissue</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3: The presence of biofilm in acquired cholesteatoma specimens.

<table>
<thead>
<tr>
<th>Description of specimen</th>
<th>The number of biofilm positive samples</th>
<th>The number of biofilm negative samples</th>
</tr>
</thead>
</table>
| Cholesteatoma middle ear mucosa | 5 (38.4%) | A:5  
S:1  
PT:1 | A:6  
S:1  
PT:1 |
| Cholesteatoma mastoid tissue | 2 (15.3%) | A:2  
S:0  
PT:0 | A:7  
S:2  
PT:2 |
| Cholesteatoma ossicle samples | 2 (15.3%) | A:2  
S:0  
PT:0 | A:7  
S:2  
PT:2 |

A: attic; S: sinus tympani; PT: pars tensa.

whether the biofilm shows a significant difference depending on the location of the cholesteatoma could not be determined.

Scanning electron microscopy demonstrated that the distribution of bacterial microcolonies was not homogenous throughout the tissue surface in biofilm-positive samples. In some areas, extracellular material was observed connecting the bacteria (Figures 1(a), 1(b), and 1(c)). Occasionally, in those samples that appeared to be negative at low magnifications, the presence of a biofilm was encountered as the magnification increased. In contrast, occasionally, samples that appeared to be positive at low magnifications showed, a rough surface structure of the tissue (Figures 2(a) and 2(b)) or erythrocytes as the magnification increased (Figures 3(a) and 3(b)). Figure 4 also indicated that the biofilm negative sample.

4. Discussion

The data presented in this study support the hypothesis that biofilms may play a significant role in otolaryngologic infections. In particular, the greater presence in patients with CSOM (7 of 10, 70%) and cholesteatoma (8 of 13, 61.5%) does suggest that the biofilms are pathogenically important. With respect to this correlation, Lee et al. [17] reported that frequency of biofilms was 60% (6 of 10) in CSOM, and Lampikoski et al. [18] reported 66% (19 of 29) in mastoid mucosa with CSOM. Therefore, Roland proposed that biofilms are the likely cause of CSOM, which would explain the observed resistance to antibiotic therapy [19]. Biofilms may attach to damaged tissue, such as ulcerated middle ear mucosa or exposed osteitic bone, and are thought to cause persistent infections [20]. In addition, the frequent and inappropriate use of topical antibiotics and antiseptic solutions in COM may create a suitable environment for microorganism resistance.

As expected, we found the presence of biofilms to be significantly higher in patients with CSOM (70%) compared with those with CNSOM (54.5%). To our knowledge, there have not been any published data regarding these two groups and biofilm conditions that can be compared with our results. Thus, these findings warrant further investigation to determine the exact role of biofilms in the pathogenesis of CSOM and CNSOM infections. Recently, the pathogenesis of acquired cholesteatoma disease has been studied extensively, but the mechanisms are not yet fully understood. Lampikoski et al. reported biofilm formation in three of four infected cholesteatoma patients and in three of five (60%) cholesteatoma cases [18]. In our study, we found results similar to those from the literature (8 of 13, 61.5%). Lampikoski et al. indicated that the cholesteatoma tissue could be hypothesized to be a beneficial substrate for biofilms to settle upon [18]. Cholele Faddis described the presence of biofilms in human and gerbil cholesteatomas and identified biofilms in 16 of 24 clinical cases (66%) [21]. The authors suggested that the bacteria can infect the keratin matrix, forming biofilms that, in turn, lead to chronic persistent infections. In our study, cholesteatoma also appeared to be an ideal environment for the development of biofilms.

Generally, the first choice for ossicle chain reconstruction in COM is to use the patient’s own ossicles [22]. However, there is a risk of cholesteatoma matrix remaining on
Figure 1: (a) Scanning electron micrograph of middle ear tissue covered with biofilm. Arrows indicate the extracellular material connected to the bacteria. The specimen was removed from a patient undergoing surgery for nonsuppurative chronic otitis media. ((b) and (c) higher magnifications of same pictures).

Figure 2: (a) Arrows indicated that the biofilm suspected regions; (b) however, in some samples, rough surface structure was seen as the magnification increased.

Figure 3: This image shows a middle ear sample surface. Specimen was taken from a patient undergoing surgery for chronic otitis media with cholesteatoma. This sample appeared to be biofilm positive showed at low magnification, but erythrocytes (arrows) were seen as magnification increased.
Biofilm detection was difficult. Occasionally, because of the sample size is too small, surveying the entire specimen for drawbacks in using this method. For example, although our identify and characterize biofilms, we have experienced some was not feasible in our study.

Infection of the upper airways. Thus, the inclusion of controls from a age-matched controls subject who has never had an infection of the upper airways. This condition may also help prevent biofilm adhesion.

The granulated tissue may be produced as a response to microbial biofilm adhesion to alloplastic materials such as tympanostomy tubes and partial or total ossicular replacement prosthesis or as a secondary consequence of bacterially induced inflammation in the middle ear. Chole and Faddis reported that recurrent infections or hypertrophy raises the possibility that the bacteria are sequestered from the host defenses [21]. In addition, hypertrophy is thought to be caused by multiple and sometimes resistant bacteria. In our study, we also determined that the biofilm rates were higher in hypertrophic and granulated tissue samples than in normal mucosa.

A limitation of the present study is the lack of a control group. Tissue from an appropriate control group is ethically problematic to obtain because it should be composed of tissue from age-matched control subjects who have never had an infection of the upper airways. Thus, the inclusion of controls was not feasible in our study.

Although SEM has been widely used by investigators to identify and characterize biofilms, we have experienced some drawbacks in using this method. For example, although our sample size is too small, surveying the entire specimen for biofilm detection was difficult. Occasionally, because of the rough topographic structure of the surface or crypts, these regions could not be examined in detail. Recently, newer techniques, such as confocal laser scanning microscopy, have also been used in biofilm research. These methods allow for further elucidation of the structure-function relationships in biofilms. However, we were unable to find any studies in the literature comparing the sensitivity and specificity of the microscopic techniques used to detect human host biofilms.

In conclusion, our research supports the hypothesis in which biofilms are involved in CSOM, cholesteatoma, and, to a lesser degree, CNSOM. In this situation, the careful use of topical or systemic antimicrobials is essential. The first choice is surgery; and, during the surgery, hypertrophic tissue must be carefully removed from the normal tissue. There are many reasons for failure after the operation. If the tissue with the potential to harbor biofilms, such as granulated tissue, cannot be cleaned sufficiently, residual biofilms may be one reason for the surgery failure.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgment

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References


Figure 4: This image shows the surface of a middle ear of a patient with chronic suppurative otitis media. The specimen was used as a control in our study. Note the relatively smooth surface and lack of organisms.


Research Article

Possible Protective Effect of Sertraline against Cisplatin-Induced Ototoxicity: An Experimental Study

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Background/Objective. Cisplatin is a widely used chemotherapeutic agent, but its ototoxicity side effect can occur in the majority of patients. Lots of agents were tried to prevent this, but there is not a routine treatment modality yet. The aim of this study was to evaluate the otoprotective effect of sertraline, which is an antidepressant with neuroprotective effects, against cisplatin, in rats.

Design. Experimental animal study.

Material and Methods. Forty-eight rats were randomly separated in two groups as groups I and II. Group I was identified as the control group and only a single dose of intraperitoneal cisplatin was administered. In group II, in addition to cisplatin, sertraline was administered to the rats through an oral cannula for ten-day period. Distortion product otoacoustic emission measurements were performed at the first day and the 10th day.

Results. When the ototoxicity rates after cisplatin in group I and group II in distortion product otoacoustic emission measurements were compared, it was statistically significantly lower in group II in frequencies of 5652, 6165, 6726, 7336, and 7996 Hz ($P < 0.05$).

Conclusion. Sertraline seems to have a protective effect on cisplatin ototoxicity and could be used to prevent the ototoxicity and also to treat the depression that occurred in cancer patients together.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum) is a widely used chemotherapeutic agent to treat a variety of soft tissue tumors. It is an effective agent especially in head and neck cancers but has various side effects such as nausea, vomiting, neurotoxicity, nephrotoxicity, vestibulotoxicity, and ototoxicity. The most important of these for otolaryngologist is of course ototoxicity, and an elevation in hearing thresholds can occur in up to 75–100% of patients [1]. In experimental studies, cisplatin is characterized with hearing loss in high frequencies (4000–8000 kHz) [2]. Wang et al. showed that giving a dose of 10 mg/kg cisplatin induces apoptosis of cochlear cells, especially in inner and outer hair cells, and stria vascularis [3]. This causes irreversible hearing loss. Patients who are treated with cisplatin are cancer patients already having difficulties in communication, and the hearing loss worsens it. Thence, the incidence of depression increases in these patients. Lots of agents were tried to prevent cisplatin ototoxicity like antioxidant agents, N-acetylcysteine, neurotrophins, p53 inhibitors, and corticosteroids [19]. But lots of these require invasive approaches to deliver the agent into the inner ear and will cause an extra stress to the cancer patients. Sertraline is a selective serotonin reuptake inhibitor used widely in depression treatment. It has neuroprotective and antioxidant effects, stimulates neurogenesis, and increases antiapoptotic protein levels [4, 5]. The aim of this study was to evaluate the otoprotective effect of sertraline, against cisplatin, in rats.

2. Material and Methods

2.1. Animals. This study was performed in Kocaeli University Animal Research Laboratory (DETAB) and was approved by the Committee for Ethics in Animal Experiments at Kocaeli University. Forty-eight female, Wistar Albino rats weighing 200 to 250 g and 2 months of age were used. All animals were housed double in standard rat cages in a controlled environment with a temperature of 20°C to 22°C and with 50% to 70% relative humidity, with a 12-hour light-dark cycle. They were fed with rat chow and water.
2.2. Experimental Design and Drug Administration. Forty-eight rats were included in the study. Both ears of all rats were examined by otomicroscope and DPOAE was performed. A total of thirty rats with SPL amplitude in either ears equal or more than 15 dB were deemed eligible for the study. Eighteen rats with SPL amplitude low than 15 dB were excluded from the study. Rats were randomly separated in two groups as groups I and II. Group I was identified as the control groups and only a single dose of intraperitoneal (IP) cisplatin was administered in a dose of 14 mg/kg to the rats in this group. In group II, sertraline diluted with distilled water to 10 mg/mL per dose through an oral cannula at 10 mg/kg/day was administered to the rats, in addition to a single dose of cisplatin at a dose of 14 mg/kg IP (Figure 1). Sertraline treatment began seven days prior to cisplatin administration and was continued for three more days after cisplatin administration; thus, administration continued for a total of ten days. Peroral sertraline and IP cisplatin applications were performed using only ether anesthesia. During DPOAE measurements in all rats, intramuscular 1 mg ketamine and 1 mL xylazine were administered for anesthesia.

2.3. Distortion Product Otoacoustic Emission Recordings. An audiologist and an audiometrist using Otodynamics Ltd. ILOv6 equipment with a minimum size rubber tympanometry probe attached to the tip of the probe of the equipment performed DPOAE measurements. DPOAE (2\(f_1\) - \(f_2\) cubic distortion product components) was performed in the General Diagnostic mode, using both DP-gram and input/output (I/O) measurements. DPOAEs were measured using stimulations of different frequency and severity. Primer stimulant severity was equalized to 65 dB in DP-gram measurements (\(L_1 = L_2\)). The two different frequencies (\(f_1\) and \(f_2\)) were arranged as \(f_2/f_1\), so that the most powerful responses would be obtained. DP-gram measurements were performed in 1001, 1501, 2002, 3003, 4004, 4358, 4761, 5188, 5652, 6154, 6726, 7336, and 7996 Hz frequencies. Noise levels for both DP-gram and I/O functions were measured at frequencies more than 50 Hz. The OAEs equal to 3 dB or more than the noise level in 2\(f_1\) - \(f_2\) frequencies were accepted as hearing is present during the measurements. If the OAE is less than 3 dB, it is accepted as hearing is absent which means that ototoxicity occurred in this ear. Responses obtained during the first round are recorded in all measurements. In group I and group II, I/O measurements were performed in each frequency for situations such as \(f_1 = f_2 = 80, 70,\) and 65/55 in 1001, 1501, 2002, 3003, 4004, 4358, 4761, 5188, 5652, 6154, 6726, 7336, and 7996 Hz prior to drug administration. Oral sertraline was given to group II for 7 days in a dose of 10 mg/kg/day. A single dose of IP cisplatin was given in both group I and group II in a dose of 14 mg/kg on day seven. Three days after cisplatin application, I/O measurements in \(f_1 = f_2 = 80, 70,\) and 65/55 for each frequencies which were 1001, 1501, 2002, 3003, 4004, 4358, 4761, 5188, 5652, 6154, 6726, 7336, and 7996 Hz were performed and recorded in surviving rats in group I and group II.

2.4. Statistical Analysis. The statistical analysis was done by Kocaeli University Public Health Department. DPOAE amplitudes were analyzed by Mann-Whitney test, and the presence of ototoxicity is analyzed by Chi-square test. Data were analyzed by using SPSS for Windows 13.0.

3. Results

Seven out of fourteen rats died three days after cisplatin administration in group I. Two of the sixteen rats in group II died due to ether inhalation during sertraline administration (before cisplatin administration), and additional two died
for reasons other than ether inhalation (on the 2nd day of cisplatin administration). On the 3rd day of cisplatin administration, I/O measurements in $f_1 = f_2 = 80, 70,$ and $65/55$ in each of the frequencies of 1001, 1501, 2002, 3003, 4004, 4358, 4761, 5188, 5652, 6165, 6726, 7336, and 7996 Hz were performed and recorded in the seven surviving rats in group I (14 ears) and in twelve surviving rats in group II (24 ears) and DPOAEs were performed.

The numbers of hearing ears with frequencies before and after cisplatin administration in groups I and II are given in Figure 2. The DPOAE amplitude averages of all groups with frequencies are given in Figure 3. When we look at Figure 3, it looks like there was a difference between groups I and 2. But, when we compare them statically with Mann-Whitney test, there were no significant differences between them.

In fourteen ears (seven rats) in group I in I/O measurements for $f_1/f_2 = 65/55$ after cisplatin administration, ototoxicity was observed in nine (64%) at frequencies of 4004, 4358, 4761, and 5188 Hz, 10 (71%) at frequencies of 5652, 6165, 6726, and 7336 Hz, and eleven (78%) at a frequency of 7996 Hz. In 24 ears (twelve rats) in group II, in I/O measurements for $f_1/f_2 = 65/55$ after cisplatin administration, ototoxicity was observed in eight (33%) at frequencies of 5188 and 5652 Hz, in nine (37%) at frequencies of 4004, 4358, 6165, and 6726, and in ten (41%) at frequencies of 4761, 7336, and 7996 Hz.

When the ototoxicity rates after cisplatin in group I and group II in I/O measurements in $f_1/f_2 = 65/55$ were compared, it was statistically significantly lower in group II in frequencies of 5652, 6165, 6726, 7336, and 7996 Hz ($P < 0.05$). No significant differences were observed in lower frequencies such as 1001, 1501, 2002, 3003, 4004, 4358, 4761, and 5188 Hz. The ototoxicity rates of two groups at the 10th day are given in Figure 4.

### 4. Discussion

There are lots of chemotherapeutic agents nowadays which have less side effects but cisplatin is still more effective than most of these agents, and so it is still being used widely. For this reason, ear nose and throat specialists frequently encounter adverse effects of cisplatin such as vestibulotoxicity and ototoxicity. Risk increases, especially in younger patients, with large cumulative doses, individuals with prior hearing loss, renal disease, or with a history of radiation to brain or skull base [6].

Cisplatin is known to produce ototoxicity through some mechanisms such as myelin sheath separation in type I spiral
ganglion cells, apoptosis induction in the organ of Corti, increased free oxygen radicals in cochlear cells. In addition, it has deleterious effects on the basal turn stria vascularis, including strial edema, bulging, rupture, and compression of the marginal cells, and depletion of organelles from the cytoplasm. Molecules preventing oxidative stress are glutathione and the antioxidant enzymes, heat shock proteins, adenosine A1 receptors, NRF2 and heme-oxygenase-1, the thiol antioxidant and the antioxidant enzymes, heat shock proteins, cytoplasm. Molecules preventing oxidative stress are glutathione and the antioxidant enzymes, heat shock proteins, adenosine A1 receptors, NRF2 and heme-oxygenase-1, the kidney injury molecule (KIM-1), and several thiol antioxidants. In addition, intratympanic dexamethasone application has also been shown to be preventive against cisplatin toxicity [6]. Perilymphatic perfusion of sodium thiosulfate in guinea pigs prevents cisplatin ototoxicity [7], whereas application to the round window membrane using an osmotic mini pump is not effective in preventing cisplatin ototoxicity [8]. N-Acetylcysteine protects against cisplatin ototoxicity whether it is administered systemically or transtympanically [6, 9, 10]. Amifostine was found to protect against peripheral ototoxicity in the hamster but also to increase neurotoxicity [11]. Other antioxidant agents D-methionine, alphatocopherol, aminoguanidine, sodium salicylate, and ebselen were also found to prevent ototoxicity of cisplatin [6, 12, 13]. A1 adenosine receptor agonist, R-PIA [14], neurotrophins such as neurotrophin-3 [15], flunarizine [16], intracochlear perfusion of inhibitors of caspase-3 and caspase-9 [3], XIAP (the X-linked inhibitor of apoptosis protein) [17], and the p53 inhibitor pifithrin-alpha [18] were also found as protective. In addition, intratympanic dexamethasone application has also been shown to be preventive against cisplatin toxicity [19]. But lots of these studies are in vitro studies, and investigators used invasive approaches to deliver the agent into the inner ear [6].

Sertraline is a selective serotonin reuptake inhibitor (SSRI) and is widely used for the treatment of patients with depression and severe anxiety disorders. It is also shown that SSRIs can also stimulate neurogenesis and protect neurons against metabolic/oxidative insults [4, 5]. Duan et al. studied sertraline and found that sertraline increases levels of brain-derived neurotrophic factor levels, preserves chaperone protein HSP70 levels and antiapoptotic protein Bcl-2 levels, restores depleted serotonin levels, retards motor behavioral impairment, and enhances neurogenesis [5]. Duan and Kumar stated enhancing effect of sertraline on neurogenesis and its antioxidant effect [5, 20].

This study especially takes into account the antioxidant, neuroprotective effects of sertraline and protective effects of antiapoptotic protein Bcl-2. The probable preventive effect of sertraline in cisplatin ototoxicity was evaluated in this study. No statistically significant differences were observed between the cisplatin group and cisplatin + sertraline group in frequencies lower than 5000 Hz while in frequencies higher than 5000 Hz, the cisplatin + sertraline group had statistically significantly better results in hearing. Sertraline in a dose of 10 mg/kg/day was administered, beginning seven days prior to cisplatin treatment and ending three days after cisplatin administration, for a total of ten days. Future studies with higher doses in longer durations might result in more protective effects or similar results might be obtained with lower doses and shorter durations. Particularly if lower doses yield successful results, a suggestion could be made for clinical practice such as administering low dose sertraline to patients who are taking cisplatin with risk factors for toxicity.

Some invasive methods such as intratympanic steroid application have also been demonstrated to be effective in preventing ototoxicity. However, it is difficult to apply these methods in patients already receiving a lot of invasive or noninvasive treatments because of their malignant diseases. These additional procedures might cause additional anxiety for the patient that would result in compliance problems in clinical practices. Therefore, using easily tolerated and orally administered agents with no adverse effects, such as sertraline, would be advantageous.

It is not practical to suggest sertraline use in all patients using cisplatin to prevent ototoxicity. However, if the hearing and communication are important tasks from the perspective of the patients with risk factors, its use can be suggested under guidance of future clinical studies. In addition, if depression is present in patients taking cisplatin and medical treatment is needed for depression, sertraline could be the drug of choice because of its decreasing effect of ototoxicity. The authors of this study plan to run a clinical study to compare the hearing thresholds of patients taking cisplatin and sertraline for their depression with a control group.

5. Conclusion

Cisplatin has been widely used in spite of its ototoxicity, which is important for the specialty of ear, nose, and throat, and all other adverse effects. When the increase in the number of new cancer cases and quality of life are taken into account, agents that would decrease the ototoxic effects of drugs like cisplatin should be evaluated and used. For this reason, sertraline was used in this study, and it was determined...
that sertraline would be beneficial to treat depression, could improve communication problems, and also could decrease hearing loss due to ototoxicity. Future clinical and experimental studies would be enlightening.

**Conflict of Interests**

The authors declare that there is no conflict of interests.

**References**


Clinical Study

Extended High Frequency Audiometry in Polycystic Ovary Syndrome

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Objective. Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting 5–10% of women in reproductive age. Insulin resistance, dyslipidemia, glucose intolerance, hypertension, and obesity are metabolic disorders accompanying the syndrome. PCOS is a chronic proinflammatory state and the disease is associated with endothelial dysfunction. In diseases with endothelial damage, hearing in high frequencies are mostly effected in early stages. We evaluated extended high frequency hearing loss in PCOS patients.

Material Methods. Forty women diagnosed as PCOS and 25 healthy controls were included in this study. Age and BMI of PCOS and control groups were comparable. Each subject was tested with low (250–2000Hz), high (4000–8000Hz), and extended high frequency audiometry (8000–20000). Hormonaland biochemical values including LH, LH/FSH, testosterone, fasting glucose, fasting insulin, HOMA-I, and CRP were calculated.

Results. PCOS patients showed high levels of LH, LH/FSH, testosterone, fasting insulin, glucose, HOMA-I, and CRP levels. The hearing thresholds of the groups were similar at frequencies of 250, 500, 1000, 2000, and 4000 Hz; statistically significant difference was observed in 8000–14000 Hz in PCOS group compared to control group.

Conclusion. PCOS patients have hearing impairment especially in extended high frequencies. Further studies are needed to help elucidate the mechanism behind hearing impairment in association with PCOS.

1. Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting 5–10% of women in reproductive age [1]. The etiology is unknown. Disease is characterized by oligo-amenorrhea, hyperandrogenism, and polycystic ovaries [2]. PCOS is a chronic condition beginning most commonly in adolescence. Insulin resistance, dyslipidemia, glucose intolerance, hypertension, obesity, and nonalcoholic fatty liver are metabolic disorders accompanying syndrome [3–5]. Therefore, a higher cardiovascular risk is reported [6]. Endothelial cell dysfunction is one of the earliest stages of atherogenesis. Therefore, markers reflecting endothelial injury have been searched [7]. Tumor necrosis factor, highly sensitive C-Reactive Protein, homocysteine, and Plasminogen activator inhibitor-1 are some of the cardiovascular risk markers that are increased in PCOS [8]. PCOS is a chronic proinflammatory state. An imbalance between prooxidants and antioxidants in PCOS produces an oxidative state [9]. There is also an association between inflammation at the molecular level and insulin resistance in the disorder [8, 10]. Elevations of a number of circulating proatherogenic inflammatory mediators have been independently reported in PCOS [11, 12]. Meta-analysis of the 31 articles reported that circulating CRP was 96% higher in women with PCOS compared to healthy controls [13]. The relationship between CRP and atherothrombotic cardiovascular disease, renal function abnormalities, has been reported in a number of studies [14]. Factors predisposing for endothelial injury include hyperinsulinemia, insulin resistance, dyslipidemia, and chronic low-grade inflammation which often accompanies PCOS [15].

Recently, a study conducted by Oghan et al. showed that patients with PCOS have sensorineural hearing loss in high frequencies [16]. They postulated that hyperandrogenism...
was the possible etiological factor. However, to date, the relationship between PCOS and hearing has not been fully understood. The aim of the present study was to determine the status of extended high frequency audiometry in cases of PCOS and to evaluate if there are other possible contributing factors that cause hearing loss.

2. Material Method

The study was carried out at Dumlupinar University Kutahya Evliya Celebi Training and Research Hospital gynecology outpatient clinic. Forty patients with the PCOS and twenty-five healthy subjects were enrolled in this prospective study. The control group consisted of patients who admitted to hospital for routine gynecological examination.

We included healthy women as controls with normal menstrual cycles, with no evidence of hyperandrogenism, and with normal ovarian morphology on pelvic ultrasonography. Ferriman Gallwey scores of all control patients were under 8. PCOS was defined as the presence of two of the following three features after the exclusion of other etiologies [17], (i) oligo- or anovulation (fewer than six menstrual periods in the preceding year), (ii) hyperandrogenism and/or biochemical signs of hyperandrogenism, and/or (iii) polycystic ovaries.

The study was conducted according to the guidelines for clinical studies described in the Declaration of Helsinki (as revised by the World Medical Association, http://www.wma.net/). Regional Ethical Committee approved the study. All patients gave oral and written informed consent prior to the examination.

Exclusion criteria were tinnitus, middle ear disease, diabetes mellitus, family history of hearing loss, history of acoustic trauma, conductive hearing loss, exposure to ototoxic substances, occupational noise exposure, autoimmune diseases, history of smoking, ongoing infection-inflammation, and being on any medication.

Body mass index (BMI) was calculated as weight (kg)/height (m)². Systolic (SBP) and diastolic blood pressure (DBP) were measured twice in the right arm in a relaxed sitting position. Two measurements were taken 15 minutes apart. The average of two was used.

Blood samples were collected during early follicular phase of menstrual cycle after at least 12-hour fasting. Levels of glucose, insulin, and hormone profile (follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), total and free testosterone (Total-T and Free-T), and prolactin (PRL)) were determined. Plasma glucose was determined with the glucose hexokinase method (Cobas Integra 400 Plus, Roche Diagnostics, and Mannheim, Germany). Hormone profile is measured with electrochemiluminescence assays (ELECY S 2010 HITACHI, Roche Diagnostic, Germany).

Plasma concentrations of insulin were measured by chemi-luminescent immunoassay (Immulite One; BioDPC, Los Angeles, CA, USA). Insulin resistance was measured with HOMA-IR (homeostasis model assessment for insulin resistance) [18].

All the participants were subjected to careful ear examination to identify any abnormalities that may interfere with hearing such as a perforated tympanic membrane or other middle ear pathologies. All subjects had normal immittance audiometry results. Each participant was tested with Low Frequency Audiometry (LFA); 125 Hz to 2 kHz, High Frequency Audiometry (HFA); 4 kHz to 8 kHz, and Extended High Frequency Audiometry (EHFA); 9 kHz to 20 kHz. Audiometry was performed by an expert audiologist blinded to the study.

All statistical analyses were performed using the SPSS for Windows, version 17.0. Nonparametric tests were used as the variable hearing threshold had abnormal distribution due to data dispersion and lack of distribution symmetry. After testing the normal distribution, comparisons between the groups were tested using the t-test. The chosen significance level was P < 0.05.

3. Results

Following through examination, otologic and audiometric evaluation, 40 patients with PCOS and 25 healthy controls were included in the final analysis. Two groups were comparable with regard to age and BMI. The mean age was 23.8 ± 4.6 years in PCOS group and 24.6 ± 4.8 years in control group. The mean BMIs were 25.9 ± 4.8 kg/m² and 24.4 ± 3.8. Demographical and laboratory findings are shown in Tables 1 and 2.

A significant difference in LH, LH/FSH, Testosterone, CRP, fasting glucose, fasting insulin, and HOMA index was observed among the two groups (Table 2).

The hearing thresholds for the left and right ears are described in Table 3. Although the hearing thresholds of the
Table 3: Hearing thresholds of PCOS and control group.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>PCOS (Mean)</th>
<th>Control (Mean)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 Right</td>
<td>12,9</td>
<td>11,7</td>
<td>0,907</td>
</tr>
<tr>
<td>Left</td>
<td>12,1</td>
<td>11,7</td>
<td>0,318</td>
</tr>
<tr>
<td>500 Right</td>
<td>11,2</td>
<td>8,3</td>
<td>0,117</td>
</tr>
<tr>
<td>Left</td>
<td>10,9</td>
<td>7,2</td>
<td>0,103</td>
</tr>
<tr>
<td>1000 Right</td>
<td>10,3</td>
<td>8,3</td>
<td>0,839</td>
</tr>
<tr>
<td>Left</td>
<td>10,3</td>
<td>7,2</td>
<td>0,530</td>
</tr>
<tr>
<td>2000 Right</td>
<td>9,7</td>
<td>10</td>
<td>0,532</td>
</tr>
<tr>
<td>Left</td>
<td>10,3</td>
<td>10</td>
<td>0,231</td>
</tr>
<tr>
<td>4000 Right</td>
<td>11</td>
<td>11,7</td>
<td>0,295</td>
</tr>
<tr>
<td>Left</td>
<td>11,3</td>
<td>11,7</td>
<td>0,956</td>
</tr>
<tr>
<td>8000 Right</td>
<td>22,1</td>
<td>12,8</td>
<td>0,02</td>
</tr>
<tr>
<td>Left</td>
<td>21,9</td>
<td>12,2</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>10000 Right</td>
<td>30,7</td>
<td>24,7</td>
<td>0,014</td>
</tr>
<tr>
<td>Left</td>
<td>35</td>
<td>25,8</td>
<td>0,010</td>
</tr>
<tr>
<td>12000 Right</td>
<td>43,8</td>
<td>30,6</td>
<td>0,001</td>
</tr>
<tr>
<td>Left</td>
<td>43,3</td>
<td>28,9</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>14000 Right</td>
<td>56</td>
<td>41,4</td>
<td>0,028</td>
</tr>
<tr>
<td>Left</td>
<td>63,8</td>
<td>44,2</td>
<td>0,004</td>
</tr>
<tr>
<td>16000 Right</td>
<td>77,9</td>
<td>66,4</td>
<td>0,592</td>
</tr>
<tr>
<td>Left</td>
<td>80,4</td>
<td>64,2</td>
<td>0,606</td>
</tr>
<tr>
<td>18000 Right</td>
<td>90</td>
<td>90</td>
<td>0,209</td>
</tr>
<tr>
<td>Left</td>
<td>91,7</td>
<td>92,4</td>
<td>0,727</td>
</tr>
<tr>
<td>20000 Right</td>
<td>102,5</td>
<td>102,6</td>
<td>0,772</td>
</tr>
<tr>
<td>Left</td>
<td>100,3</td>
<td>99,8</td>
<td>0,290</td>
</tr>
</tbody>
</table>

Groups were similar at frequencies of 250, 500, 1000, 2000, and 4000 Hz, significant hearing loss was observed at frequencies of 8000, 10000, 12000, and 14000 Hz in PCOS group compared to controls (Figure 1). There was no statistically significant difference in tympanometric results between the two groups.

4. Discussion

To the best of our knowledge, this is the first study evaluating extended high frequency hearing loss in women with PCOS compared to healthy women. Routine audiometry is restricted to 125–8000 Hz frequencies. However, in diseases with endothelial damage, high frequencies are mostly affected in early stages. High frequency audiometry was introduced into clinical practice in 1960s [19].

In the present study, HOMA-I, CRP, LH, LH/FSH, testosterone, fasting glucose, and insulin levels were higher in women with PCOS. CRP is a circulating marker of the proinflammatory state in PCOS as evidenced by the 2-fold elevation in circulating CRP compared to controls. Similarly, in our study CRP was significantly higher in PCOS patients (P < 0.05). A meta-analysis of the most comparable studies indicates that circulating CRP is elevated in PCOS reflective of the chronic low-grade inflammation present in the disorder. They also found that elevated circulating CRP in PCOS is independent of obesity [13]. Although we could not demonstrate a direct correlation between CRP levels and hearing thresholds, chronic inflammation may play a role in the pathogenesis of hearing impairment in PCOS. Main causes of hearing loss seen in EHF in PCOS patients may also be multifactorial.

Early signs of endothelial damage and increased cardiovascular disease risk have been previously described in PCOS patients [20]. Atherogenesis and endothelial dysfunction have been attributed to a number of biochemical and hormonal alterations in PCOS. Elevated mean platelet volume, white blood cell, D-Dimer, and androgen levels have been reported as potential indicators of risk factor for atherogenesis [21, 22].

EHF with determination of air conduction thresholds in the frequency range of 8–20 kHz has been used in a number of studies in recent years [23]. Sensorineural hearing loss has been reported in a number of chronic systemic inflammatory disorders such as ankylosing spondylitis and rheumatoid arthritis, but exact cause of underlying pathology is still unknown [24, 25]. However, immunomediated vasculitis in the inner ear and ototoxic medication used for treatment was postulated as causative agents in some. Hearing losses in autoimmune diseases are reported to be mediated by vascular mechanisms [26].

Oghan and Coksuer firstly described high frequency (4000–8000 Hz) hearing loss in PCOS patients. They attributed this to the hyperandrogenism seen in the syndrome [16]. In the present study, we evaluated the hearing thresholds in EHF (8000–20000 Hz). We observed statistically significant difference in hearing thresholds between PCOS and control groups in 8000, 10000, 12000, and 14000 Hz. There were no significant differences in hearing thresholds comparing right to left ears at all frequencies (250–20000 Hz).

Despite certain limitations of our study, the results might be as preliminary findings for designing future studies on
larger populations. A limitation of this study is small population, but strength of this study is evaluating the EHFA in all participants.

High frequencies are more sensitive to vascular damage caused by the disease. Insulin resistance, hyperandrogenemia, and elevated serum CRP as an inflammatory marker in PCOS could be the cause of hearing loss in especially extended high frequencies. Our data suggest that HFA and EHFA are more efficient in detecting early hearing loss compared to pure tone audiometry. Further studies are needed to help elucidate the mechanism behind hearing impairment in association with PCOS and to see whether the impairment of EHFA in these cases is progressive. It might be possible to prevent progression of hearing impairment by revealing underlying factors.

References


Research Article

The Role of Thiamine Pyrophosphate in Prevention of Cisplatin Ototoxicity in an Animal Model

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Objective. The aim of this study was to evaluate the effectiveness of thiamine pyrophosphate against cisplatin-induced ototoxicity in guinea pigs. Materials and Methods. Healthy guinea pigs (n = 18) were randomly divided into three groups. Group 1 (n = 6) received an intraperitoneal injection of saline solution and cisplatin for 7 days, group 2 (n = 6) received an intraperitoneal injection of thiamine pyrophosphate and cisplatin for 7 days, and group 3 (n = 6) received only intraperitoneal injection of saline for 7 days. The animals in all groups were sacrificed under anesthesia, and their cochleas were harvested for morphological and biochemical observations. Results. In group 1, receiving only cisplatin, cochlear glutathione concentrations, superoxide dismutase, and glutathione peroxidase activities significantly decreased (P < 0.05) and malondialdehyde concentrations significantly increased (P < 0.05) compared to the control group. In group 2, receiving thiamine pyrophosphate and cisplatin, the concentrations of enzymes were near those of the control group. Microscopic examination showed that outer hair cells, spiral ganglion cells, and stria vascularis were preserved in group 2. Conclusion. Systemic administration of thiamine pyrophosphate yielded statistically significant protection to the cochlea of guinea pigs from cisplatin toxicity. Further experimental animal studies are essential to determine the appropriate indications of thiamine pyrophosphate before clinical use.

1. Introduction

Cisplatin is a mainstay chemotherapy drug in the treatment of a variety of solid tumors, notably testicular cancer. It is also used in the treatment of pediatric malignancies such as medulloblastoma and osteogenic sarcoma [1]. Cisplatin is cell cycle unspecific and is often used as a part in combination treatment. It has a toxic profile that is different from other important cytotoxic drugs. High doses cause nephrotoxicity, gastrointestinal toxicity, neurotoxicity, and ototoxicity, where the two latter side effects can be dose limiting even with modern preventive measures [2].

Inner ear toxicity is often a dose limiting side effect that hampers optimal cisplatin-based chemotherapy. It is normally manifested as a sensorineurural hearing loss beginning in the high frequencies, successively progressing towards the speech frequency range [3]. It is often accompanied by transient or permanent tinnitus. Sometimes these problems can be severe, and ototoxicity and vestibular toxicity are usually irreversible [4].

Cisplatin ototoxicity has several characteristics. In man it is mainly evident in the basal turn of the cochlea as degeneration of the outer hair cells (OHCs) and to some extent the inner hair cells (IHCs) and associated nerves [5]. It has been shown that the toxic effect of cisplatin may result in a degeneration of the vestibular organs as well [6], although it is rarely diagnosed. Under experimental conditions, toxicity is normally manifested among the OHCs and in the stria vascularis. Histological alterations have also been observed among the spiral ganglion cells in the guinea pig [7].
Thiamine pyrophosphate (TPP) is the biologically active form of thiamine (vitamin B₁), and it is an essential cofactor in all living systems. Microorganisms either synthesize TPP via de novo biosynthesis pathways or take exogenous thiamine from the environment via specific transporters. TPP plays a critical role in the carbohydrate and energy metabolism. In addition, TPP is involved in the α-oxidation of 3-methyl-branched and straight chain 2-hydroxy long chain fatty acids pathway functioning as coenzyme for peroxisomes. As a result TPP is a crucial cofactor for energy metabolism, antioxidation, and myelinization of nerve cells [21].

Continued high-dose cisplatin chemotherapy necessitates the investigation of strategies to decrease the dose-limiting ototoxicity. Lowering the dose intensity would not be a preferred option because this might reduce the efficacy of cisplatin. The aim of this study was to investigate the potential protective effect of TPP against the toxicity caused by cisplatin in the inner ear. This is the first publication, to our knowledge addressing the administration of TPP for cisplatin induced ototoxicity.

2. Materials and Methods

2.1. Chemicals. Cisplatin (cisplatinum, Ebewe, 0.5 mg/mL) was obtained from Liba Drug Company, Turkey. Thiamine pyrophosphate was obtained from SIGMA, Germany. All of the chemicals were of the highest quality commercially available.

2.2. Animals. Eighteen healthy adult male albino guinea pigs weighing 1200–1500 g (Erzurum Ataturk University Animal Laboratory, Turkey) were used in the current study. They had free access to water and food. The animals were kept under standard laboratory conditions, housed in a room at 20°C temperature, and 12 h light/dark cycle. This study was performed with the approval from the Ataturk University Animal Care and Use Committee.

2.3. Experimental Design. Guinea pigs were randomly divided into three groups and treated as follows: group 1 (n = 6) received an intraperitoneal (IP) injection of saline solution and cisplatin (5 mg/kg) for 7 days, group 2 (n = 6) received an IP injection of TPP (25 mg/kg) and cisplatin (5 mg/kg) for 7 days, and group 3 (n = 6) received only IP injection of saline for 7 days (employed as control group).

The animals in all groups were sacrificed under anesthesia (25 mg/kg thiopental sodium) and their cochleas were harvested for morphological and biochemical observations. All surgical procedures were performed under a dissecting microscope with sterilized instruments.

2.4. Biochemical Determination. The level of endogenous antioxidant glutathione (GSH), the activities of antioxidant enzymes glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), and the concentration of malondialdehyde (MDA), the end product of lipid peroxidation, were determined enzymatically with the techniques explained by the literature [8–11].

2.5. Histological Evaluation. In order to avoid cell destruction by autolysis or bacteria and to preserve tissue morphology and composition, the cochleas were fixed in 10% neutral buffered formalin for 24 h at +4°C temperatures. Subsequently, decalcification was achieved by submerging the samples in 10% EDTA at room temperature for 7 days. The specimens were then washed with tap water and fixed again in 10% neutral buffered formalin for 24 h.

Afterwards, the specimens were embedded in paraffin and then mounted in order to obtain mid-modular plane cuts. Sections of 5 μm of thickness were collected on glass slides and stained with haematoxylin and eosin staining. Sections were examined using a light microscope (Olympus BX 51, Japan) and digital images were obtained by a digital camera (Olympus DP 71).

2.6. Statistical Analysis. The SPSS statistical software, version 13.0 was used for the statistical analysis. Significance of the difference between the groups and subgroups were analyzed using the one way ANOVA test and Fisher’s post hoc least significant differences (LSD). A difference was deemed to be significant at P < 0.05.

3. Results

3.1. Biochemical Determination. There was a significant difference in level of GSH and MDA measurement and the activities of antioxidant enzymes GSH-Px and SOD among the groups (P < 0.05). Cochlear GSH concentrations, SOD and GSH-Px activities significantly decreased (P < 0.05) and MDA concentrations significantly increased (P < 0.05) in group 1, receiving only cisplatin, compared to the control group. In group 2, receiving TPP and cisplatin, the concentrations of GSH, MDA, and the activities of SOD and GSH-Px were near those of the control group. TPP restored the concentrations GSH and MDA and yielded statistically significant improvements in enzymatic activities of SOD and GSH-Px (P < 0.05) (Figure 4).

3.2. Histological Evaluation. Samples obtained from the guinea pigs receiving only IP saline solution (group 3), revealed normal microarchitecture of the organ of Corti (Figure 1(a), H&E; ×1000), spiral ganglion neurons (Figure 1(b), H&E; ×400) and stria vascularis without changes (Figure 1(c), H&E; ×400).

Guinea pigs receiving the IP injection of cisplatin, we found an extensive loss of the normal microarchitecture of the organ of Corti; severe destruction of the outer hair cells (Figure 2(a)); scattered spiral ganglion neurons with cell changes, such as lack of nucleus, vacuolation of the cytoplasm, and partial detachment of the myelin sheath (Figure 2(b)); generalized change in the stria vascularis, including edema of stria and shrinkage of intermediate cells (Figure 2(c)).

On the other hand, guinea pigs receiving cisplatin and TPP exhibited preserved morphology of the tunnel of Corti and outer hair cells (Figure 3(a)), no destruction of spiral ganglion cells (Figure 3(b)), and no destruction of stria vascularis (Figure 3(c)).
Figure 1: Normal microarchitecture of the organ of Corti, and (↑↑) OHCs can be seen (Figure 1(a), H&E; ×1000), spiral ganglion neurons (Figure 1(b), H&E; ×400), and stria vascularis without changes (Figure 1(c), H&E; ×400).

Figure 2: Showing extensive loss of the normal microarchitecture of the organ of Corti, severe destruction of the outer hair cells (↑↑). (b) Showing decreased number of spiral ganglion neurons with cell changes (star), such as vacuolation of the cytoplasm (↑). (c) Showing shrinkage of the intermediate cells (↑), edema of stria, and swelling of the epithelial cells of Reissner’s membrane (↑↑).

4. Discussion

Cisplatin has a potent antitumor activity against several tumors, including germ cell, ovarian, lung, head, and neck cancers, but has dosage-limiting side effects (e.g., ototoxicity and neurotoxicity) [12]. Cisplatin ototoxicity leads to a bilateral and irreversible sensorineural hearing loss that is progressive from higher to the lower frequencies. It quickly binds DNA and proteins and thereby inhibits their functions. Once bound, cisplatin induces the generation of reactive oxygen species (ROS) that interfere with the antioxidant protection of the inner ear. This event may be a trigger for apoptosis and therefore decreases the number of cells in the cochlea [12, 13].

Cisplatin have three important tissue targets in the cochlea, including organ of Corti, spiral ganglion cells, and lateral wall (stria vascularis and spiral ligament). In guinea pigs that received consecutive cisplatin applications, destruction of outer hair cells and myelin sheath detachment of spiral ganglion cells were observed [14]. Furthermore depletion of glutathione and antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and glutathione reductase) with an increase in malondialdehyde levels, an indicator of lipid peroxidation, were demonstrated in cochlear tissue samples from animals receiving cisplatin [15].

Numerous studies suggest that cisplatin induced hearing loss is mostly related with generation of ROS, initiating cascade oxidative mechanisms. Despite the presence of endogenous antioxidant molecules, including glutathione and the antioxidant enzymes, cisplatin induced oxidative stress can overwhelm these intrinsic defense mechanisms. Consequently, exogenous administrations of antioxidants have been the primary focus in the treatment of cisplatin induced ototoxicity [13].

At present, the only way to prevent cisplatin-induced ototoxicity is a limitation of the total dose per cycle, the cumulative dose, and the dose intensity [16, 17]. Obviously, this might reduce the efficacy of this cytotoxic agent. Therefore, there is a need to find effective protective drugs that prevent
cisplatin-induced ototoxicity. Even though there have been studies with multiple otoprotective agents [18–20], none of these agents have been found to be unequivocally beneficial in preventing cisplatin ototoxicity, and no agent is currently recommended for routine use.

TPP is the biologically active form of thiamine (vitamin B<sub>1</sub>), upon entry into cells, thiamine is quickly converted to TPP that is the active substance. However, recent studies indicate that mammalian peroxisomes do contain TPP but that no pyrophosphorylation of thiamine occurs in these organelles, implying that thiamine must enter the peroxisome already pyrophosphorylated [21, 22]. That is why we preferred TPP rather than thiamine in this study. TPP as an antioxidant has been investigated in the treatment of several oxidative processes; however, it has not been previously evaluated for its potential protective effect against cisplatin ototoxicity. This present study showed that TPP was protected against cisplatin induced degeneration of cochlea, stria vascularis, and spiral ganglion cells. TPP also reduced the content of MDA and increased the cisplatin-mediated decrease in antioxidative enzymes (GSH-Px, SOD) and GSH levels. These results suggest that the antioxidant defense mechanisms of the cochlea were potentiated by this treatment.

In many forms of ototoxicity, pharmacological activation of intrinsic defense mechanisms could be helpful. Cisplatin induced ototoxicity is a special issue in that the ototoxic insult is predictable. It should be possible to administer protective agents at precisely timed intervals before the insult. The results of this present study suggest that TPP is beneficial in reduction of experimental cisplatin ototoxicity in guinea pigs, and it may be a potential candidate drug in human beings.

We have demonstrated the efficacy of systemic administration of thiamine pyrophosphate in the prevention of cisplatin induced ototoxicity using a guinea pig model. Further experimental animal studies are essential to determine the appropriate indications and dosages of TPP before clinical use.

**Conflict of Interests**

The authors declare that they have no conflict of interest.

**Acknowledgment**

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**References**


